THE EFFECT OF RADIATION FROM SMALL AMOUNTS OF P^{32}, S^{35} AND K^{42} ON THE DEVELOPMENT OF ARBACIA EGGS[1]

JAMES W. GREEN AND JAY S. ROTH

Department of Physiology and Biochemistry, Rutgers University, New Brunswick, New Jersey; William Goldman Isotope Laboratory, Hahnemann Medical College, Philadelphia, Pennsylvania, and the Marine Biological Laboratory, Woods Hole, Massachusetts

In the recent extensive use of isotopes in biological research, little concern has been manifested for the effects of trace amounts of radioactive isotopes on cellular processes. Radioactive tracer studies in whole animals are usually not controlled at the level they seek to elucidate and few studies involving single cells have been designed to determine minimum levels of radioactivity which affect synthetic and developmental processes. Kamen (1947) states (p. 125), "It must be emphasized that tracer radiations in themselves constitute an ever present hazard in research on metabolism because marked physiological effects invalidating conclusions can arise if high tracer concentrations are used." Unfortunately, as he points out, little systematic information on the effects of low radiation dosage is available and what knowledge exists indicates marked variability in sensitivity in different forms. The dose range to produce various biological effects varies from 10 to 10^6 roentgens (Patt, 1953).

Thus, although our knowledge of radiation effects is large (see Henshaw, 1944; Lea, 1947; and Gray, 1952), little attention has been devoted to a study of the effects of ionizing radiations emitted from trace amounts of radioactive isotopes upon single cells. That small amounts of radiation may have significant effects on *Paramecium* has been demonstrated for x-radiation by Hance and Clark (1925), and more recently for β-radiation by Daniel and Park (1953). Packard (1916) reported acceleration of cleavage in *Arbacia* treated with short exposures to radium.

Mullins (1939), working with *Nitella*, found that concentrations of Na^{24} above one microcurie (μC) per ml. decreased the amount of ion penetration into these cells. Brooks (1943) determined the uptake of P^{32} and Na^{24} in *Arbacia* and *Asterias* eggs. His P^{32} concentrations ranged from 0.045 to 0.29 μC per ml. and because the amount of β-radiation emitted by the P^{32} was less than that observed by Mullins (1939) to affect *Nitella* permeability, he assumed that the radiation was not a factor in his measurements. In a later study (Brooks and Chambers, 1948) the P^{32} uptake in several species of Pacific coast sea urchins was investigated using concentrations of 0.001 to 0.3 μC per ml.

The present study was undertaken to determine the dosage levels of P^{32}, S^{35} and K^{42} upon eggs or sperm which would have an effect on the cleavage and subsequent development of *Arbacia* eggs. The results indicate that radiation from

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P^{32}, S^{35} and K^{42} at dose levels ranging from 0.25 to 50 μC per ml. (which includes the range used by others for permeability studies) has an accelerating or retarding effect on the first cleavage, depending upon the dose, and also retards subsequent development of the plutei.

Methods

Eggs and sperm of *Arbacia punctulata* were prepared in the manner described by Blum and Price (1950).

The isotopes used in this study were S^{35}, P^{32} and K^{42}.[2] Sea water solutions of each of these were prepared by evaporating carrier-free isotope solutions to dryness and dissolving the isotope residue in sea water. The pH of such solutions was the same as that of sea water. Stock solutions of S^{35} were prepared weekly in concentrations of 0.5, 5.0 and 50 μC per ml. P^{32} solutions were prepared in the same concentrations and used within two days. The K^{42} solutions were prepared in a concentration of one μC per ml. and used immediately.

The experiments were performed in the following manner: four 25-mm. stender dishes were placed in running sea water to a depth of one cm. to keep the contents at the temperature of the sea water. To dish one was added one ml. of filtered sea water; to dishes two, three and four were added one ml. of filtered sea water containing isotope concentrations of 0.5, 5.0 and 50 μC per ml., respectively.

When the object of the experiment was the irradiation of sperm, one or two drops of dilute sperm suspension were added to each of the dishes. Generally after two hours, one ml. of freshly washed eggs was then added, the time noted, and samples of the cleaving eggs taken at two-minute intervals for 14 minutes after cleavage began in the control. One-drop samples were preserved in 5 per cent formalin in sea water in shell vials for later counting. The per cent cleavage for a given time interval was determined by counting 100–200 eggs in each sample period. Egg irradiation experiments were performed in the same manner as the sperm experiments. One ml. of a dilute suspension of freshly washed eggs was added to each dish. Such additions diluted the isotope concentrations by one half so that eggs were exposed to 0.25, 2.5 and 25 μC of radioactivity per ml. Doubling the concentration of the eggs had no effect on the results. All experiments were carried out in duplicate. The temperatures of the control and experimental solutions varied from 22° to 24° C. but did not vary more than one half degree C. during any one experiment.

Irradiated sperm were always fertilized with non-irradiated eggs and irradiated eggs with non-irradiated sperm. No experiments were performed in which irradiated eggs were fertilized with irradiated sperm. After preserving samples of the first cleavage, the remaining zygotes in the dishes were permitted to develop. When control forms had reached the pluteus stage, usually after 48–72 hours, samples of all dishes were preserved in formalin sea water for comparison. It should be noted that after fertilization the developing larva remained in the same isotope environment as the irradiated gametes.

[2] The isotopes used in the study were obtained on allocation from the Isotopes Division, United States Atomic Energy Commission. The authors would like to acknowledge the helpful assistance of Mr. S. Berman in preparation of the isotope solutions.

Results

Preliminary experiments utilizing P^{32} had been previously carried out and reported upon (Green and Roth, 1950), but the data reported here were obtained during the summer of 1953.

Figure 1 is a plot of the per cent first cleavage against time of *Arbacia* eggs fertilized with sperm which had been irradiated for two hours prior to fertilization by three dose levels of S^{35}. The graph shows that the time of cleavage is delayed

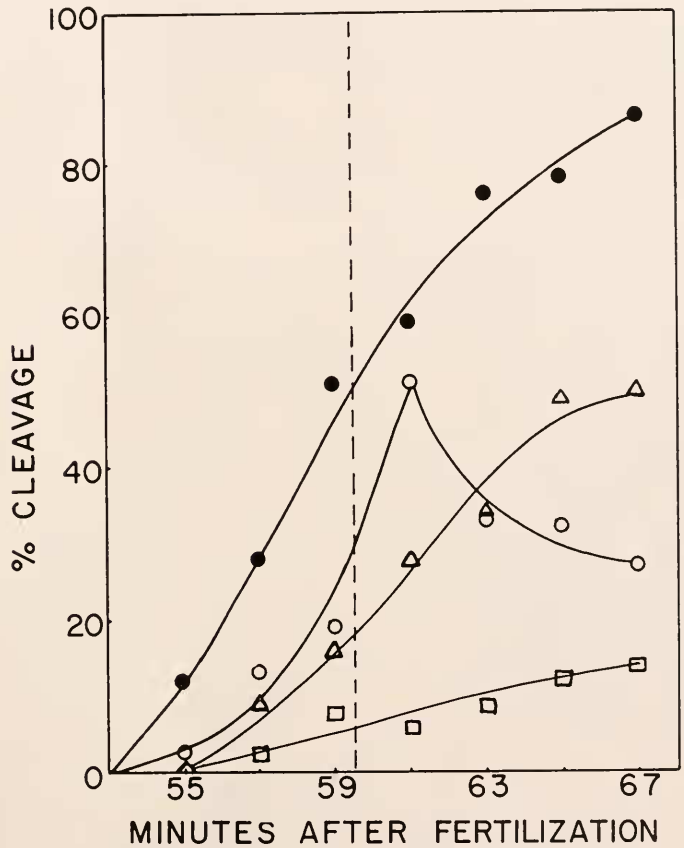

Figure 1. Plot of per cent first cleavage against time when sperm were irradiated for two hours prior to fertilization of non-irradiated eggs. ● = non-irradiated; ○ = 0.5 μC per ml. S^{35}; △ = 5.0 μC per ml. S^{35}; □ = 50 μC per ml. S^{35}.

by prior irradiation of the sperm, roughly in proportion to the dosage of radioactivity. The decrease in per cent cleavage in the case of the 0.5-μC dose after 61 minutes does not represent an error in counting but is probably attributed to experimental variation.

Figure 2 is a plot similar to Figure 1 and shows the results of irradiation of eggs for two hours with three dose levels of S^{35} prior to fertilizing with non-

irradiated sperm. Although the dose levels shown in Figure 2 are different from those of Figure 1, there is some overlapping and one can readily see that egg irradiation prior to fertilization has a less marked retarding effect on subsequent cleavage than does the irradiation of sperm. Figure 2 shows that the lowest dose level had an accelerating effect on cleavage which may not be too significant. The vertical dotted line appearing in both Figures 1 and 2 indicates the per cent

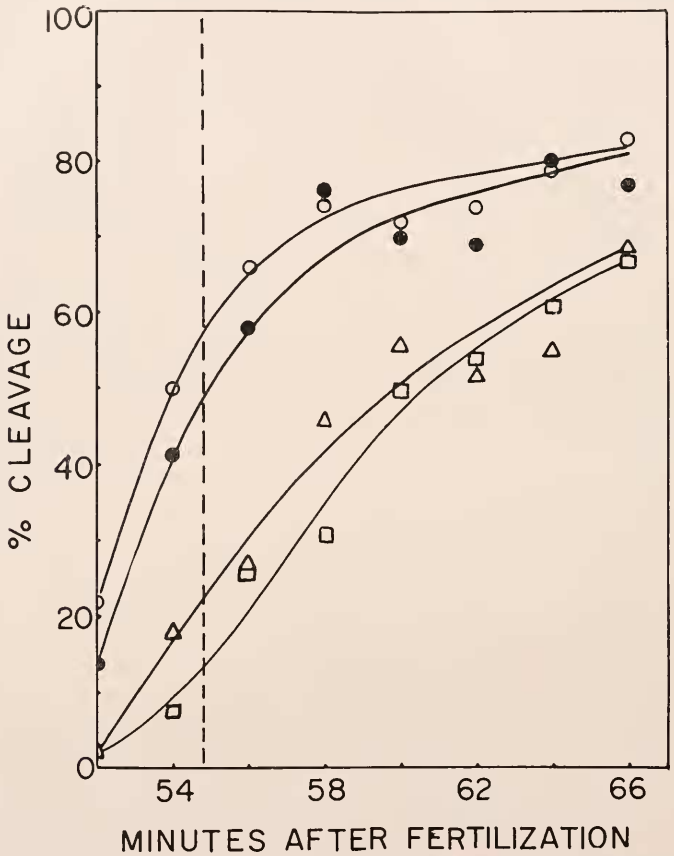

FIGURE 2. Plot of per cent first cleavage against time when eggs were irradiated for two hours prior to fertilization with non-irradiated sperm. ● = non-irradiated; ○ = 0.25 μC per ml. S^{35}; △ = 2.5 μC per ml. S^{35}; □ = 25.0 μC per ml. S^{35}.

cleavage attained by the experimental cells when the control cells had reached 50 per cent cleavage.

The data presented in Table I were obtained from graphing the results of a number of experiments in the manner of Figures 1 and 2. The amount of retardation or acceleration of cleavage by the irradiation of unfertilized gametes is presented as the per cent cleavage of the experimentally treated eggs at that time when 50 per cent of the control eggs had cleaved. This method of expressing the results was chosen because most of the egg suspensions treated with the higher

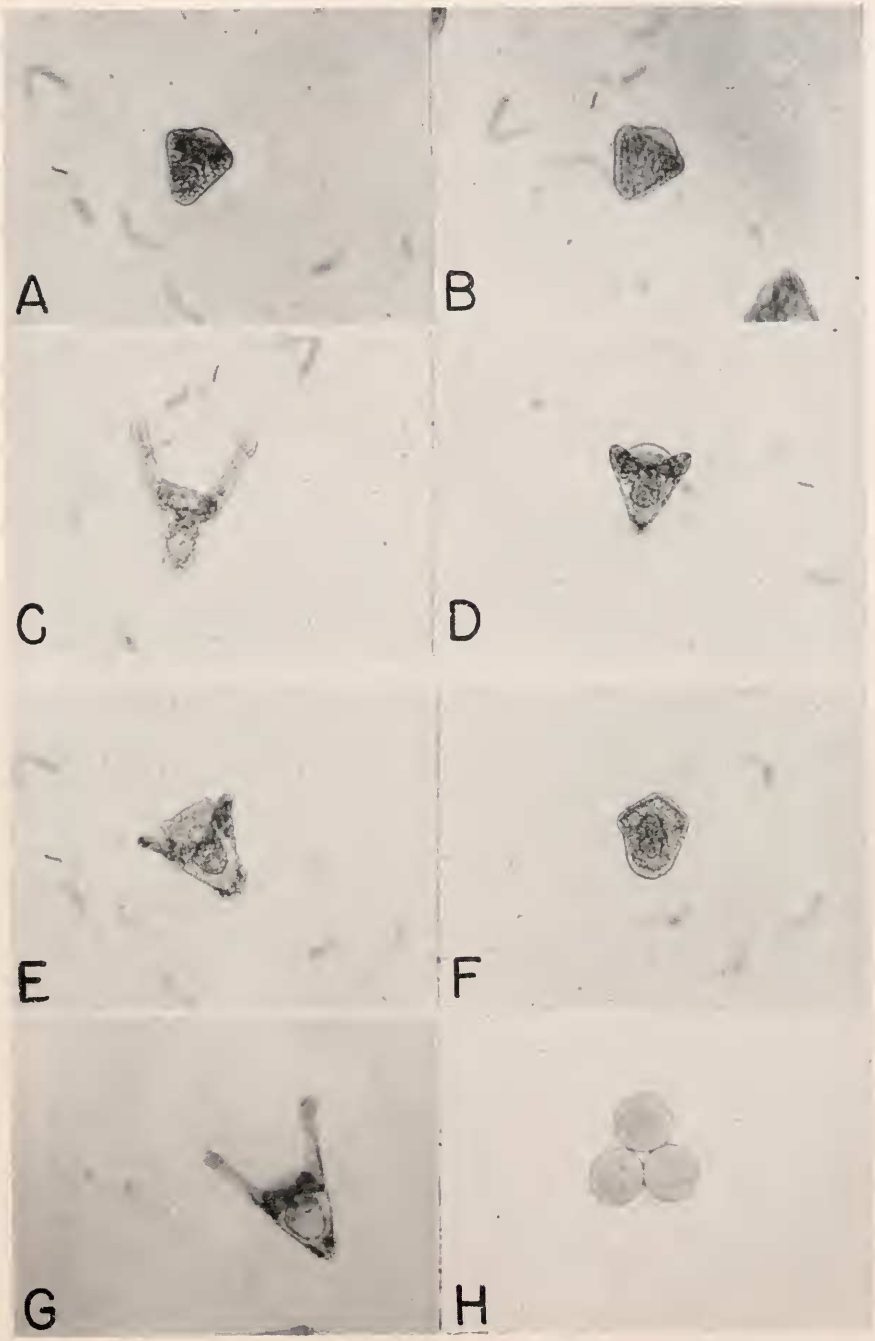

FIGURE 3. For explanation see text under "Results."

radiation doses did not attain 50 per cent cleavage during the period of observation. In general, duplicate experiments with eggs and sperm from different female and male *Arbacia* are given in the table. And, although the results from such experiments show variation, the direction and order of magnitude of the effects were frequently duplicated.

Figure 3 shows a series of photographs of developing larvae of *Arbacia* in which the effects of sperm and egg irradiation are compared. Photographs A, C, E and G are of non-irradiated cells and should be matched with photographs B, D, F and H, respectively, of corresponding irradiated cells. Egg irradiation is shown by photograph B (0.25 μC per ml. of S^{35}) and F (0.25 μC per ml. of P^{32}). In neither case are the plutei strikingly different from their corresponding

TABLE I

Effect of ionizing radiations on Arbacia egg cleavage

Date	Exp. No.	Isotope	Gamete irrad.	Prefert. expos. hrs.	Per cent of experimental eggs cleaved when 50% of controls have cleaved						
					Exposure dosage—microcuries/ml.						
					0.25	0.5	1.0	2.5	5.0	25.0	50.0
8/29/53	14A	P^{32}	Egg	2	60			43		27	
8/29/53	14B	P^{32}	Egg	2	55			50		15	
8/28/53	13	P^{32}	Sperm	2		14			0		0
7/22/53	5A	S^{35}	Egg	0	61			40		74	
7/13/53	2A	S^{35}	Egg	1	55			47		40	
7/13/53	2B	S^{35}	Egg	1	40			40		25	
7/17/53	4A	S^{35}	Egg	2	60			21		15	
7/17/53	4B	S^{35}	Egg	2	67			60		63	
7/24/53	6A	S^{35}	Sperm	2		25			15		5
7/24/53	6B	S^{35}	Sperm	2		32			13		6
8/22/53	9B	K^{42}	Egg	2		30					
8/21/53	10A	K^{42}	Sperm	2			10				
8/21/53	10B	K^{42}	Sperm	2			14				

controls. However, the effect of P^{32} was somewhat greater, as might be expected from the greater energy of its β-radiation. Sperm irradiation is shown by photographs D (0.5 μC per ml. of S^{35}) and H (0.5 μC per ml. of P^{32}). Both photographs indicate an appreciable retardation in growth as compared with corresponding controls (photographs C and G). These pictures support the data in Table I in showing that egg irradiation prior to fertilization has a less marked effect on subsequent cleavage and development than sperm irradiation.

DISCUSSION

It is rather difficult to calculate the exact exposure in terms of roentgens or equivalent roentgens (e.r.) of an individual egg or spermatozoan. The total

radiation dose in the solution during two hours, when radiation equilibrium obtains, may be calculated by the methods of Marinelli, Quimby and Hine (1948). For concentrations of 0.5, 5.0 and 50 μC of P^{32} per ml. this would be 1.74, 17.4 and 174 e.r., respectively, and for S^{35}, 0.139, 1.39 and 13.9 e.r., respectively. However, because of the small volume and thickness of solution used, the condition of "radiation equilibrium" was not fulfilled (especially in the case of P^{32}) and therefore the doses actually received by the eggs or the sperm were considerably less than those given above. This is particularly true in the case of the eggs, which lay at the bottom of the dishes and consequently were irradiated essentially from one side. In the case of the developing larvae in the presence of the higher concentrations of isotope for from 48 to 72 hours, the radiation dose might be appreciable, however. Daniel and Park (1953) found that solutions of S^{35} delivering from 0.77 to 3.13 e.r. per hour under radiation equilibrium conditions, stimulated *Paramecium* division. Their dose levels are well within the range of activities observed by us to be stimulatory to the *Arbacia* egg.

The date reported by Table I clearly show that cleavage delay results on irradiation of sperm with low levels of β-radiation. This finding is supported by the work of Mitueo *et al.* (1939) on eggs and sperm of *Pseudocentrotus depressus* exposed to β-rays from radium emanation, and was found to be true for *Arbacia* gametes irradiated with x-rays (Henshaw and Francis, 1936). Both Henshaw and Mituo irradiated testicular mashes and their results are subject to possible effects of so-called "necrohormones" (Heilbrunn and Young, 1935). Our studies were performed, however, on quite dilute sperm suspensions so that the indirect effects observed by Heilbrunn are not applicable.

The effects of low dose levels of β-radiation upon *Arbacia* zygotes and developing embryos cannot be determined by the results of Table I or Figure 3. The retardation of cleavage from sperm irradiation is continued into the pluteus stage but as all zygotes (egg-irradiated ones also) remained in the radiation environment of the gametes, the effects of gamete irradiation and zygote irradiation cannot be separated. One possible exception to this is the experiment performed in which *Arbacia* plutei were obtained after K^{42} irradiation of gametes. In this case because of the short life of this isotope, radiation levels could be expected to fall well below injury levels before plutei were formed. Egg irradiation had no effect on plutei formation in this experiment, while sperm irradiation resulted in the development of no plutei, although some initial cleavage occurred.

The knowledge that trace dose levels of these isotopes may cause biologically abnormal effects in *Arbacia* cannot be readily transferred to other types of cells. But this knowledge does carry the implication that rapidly dividing cells in the presence of radioisotopes, or, more important, cells which accumulate isotopes, may be modified in a subtle manner by the radiation emitted by these isotopes, even though the particular process studied by the investigator does not appear to be altered. A greater awareness of potential cell damage from radioactive tracers should lead to further investigations and to more systematic knowledge of limitations of these tools in biological research.

The authors would like to acknowledge the helpful assistance of Miss Mary Hodge in performing the egg counts.

Summary

Arbacia gametes (eggs or sperm), exposed to radiation from S^{35}, P^{32} and K^{42} at dose levels ranging from 0.25 to 5.0 μC per ml. and subsequently fertilized with non-irradiated gametes, formed zygotes whose first cleavage was accelerated or retarded, depending upon the dose, and whose further development was slowed at the higher dose levels. Some experimental exceptions to these findings are reported and discussed. The implications of the results for biological tracer methodology are stated.

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